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**BULLETIN No. 42** 

# VANILLA CURING AND ITS CHEMISTRY

By

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<sup>&</sup>lt;sup>1</sup> In cooperation with the Government of Puerto Rico.

### FEDERAL EXPERIMENT STATION

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UNITED STATES DEPARTMENT OF AGRICULTURE MAYAGUEZ, PUERTO RICO

# **BULLETIN NO. 42**

Washington, D. C.

October 1944

# VANILLA CURING AND ITS CHEMISTRY

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Puerto Rico Experiment Station

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#### INTRODUCTION

The characteristic flavor and aroma of vanilla beans of commerce (Vanilla fragrans (Salisb.) Ames) develop as a result of changes that take place in the beans during the curing process. Uncured pods are odorless; if left to ripen on the vine, they gradually turn from green to yellow, split open and change to dark-brown or chocolate color. The pods develop aroma as they darken, but if left on the vine they become dry and brittle and finally almost odorless. The object of curing is to arrest the natural vegetative processes and hasten the changes that lead to the formation of the aromatic flavoring constituents in the beans.

Curing consists of an initial killing and wilting treatment, followed by heating until the beans acquire the proper texture and flexibility. This is followed by drying in air to the desired moisture content, and finally by a conditioning treatment lasting at least 3 months. As curing progresses the aroma of vanilla starts to develop, but it is only toward the latter part of the conditioning period that the full aroma is manifested.

Studies on vanilla curing and the chemistry of the processes involved were first undertaken by the Puerto Rico Experiment Station of the United States Department of Agriculture in 1938 with funds provided by the Government of Puerto Rico. The Bureau of Chemistry and Soils of the United States Department of Agriculture cooperated in the project by sending staff members to Puerto Rico on three occasions to work at the station.

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#### VANILLA CURING

#### METHODS

In an experiment designed to compare several important commercial vanilla-curing methods with others recently devised at this laboratory, seven killing procedures followed by each of two sweating methods were tested.<sup>2</sup>

The killing procedures were: Holding in an oven at 60° C. for 24 hours, Mexican method (7, pp. 92-95; 13); placing in the sun for 5 hours, Mexican method (7, pp. 90-92); three 10-second immersions in water at 80° at 30-second intervals, a modification of the Bourbon method used in Madagascar and Comores (7, pp. 96-104); one 3-minute immersion in water at 65°, Bourbon method (7, pp. 96-104); exposure to ethylene gas 1:10,000 for 16 hours, an experimental method used in this laboratory (11, pp. 14-18; 14); three longitudinal scratches with a pin made over the full length of the bean, Guadeloupe method (7, p. 115); and freezing for 40 hours, followed by thawing for 2 hours, a method developed at this station (6). The time recommended for the freezing treatment is only the few hours required to freeze the beans solid, but in this case the beans were placed in a refrigerator which was opened often and the time required for freezing solid was 40 hours.

The two sweating methods were: (1) Exposure to the sun from 9 a. m. to 3 p. m. daily until flexibility was obtained, the beans, when not in the sun, being kept in blankets during the first 5 days and on racks during the remainder of the period, the method generally used in Mexico, Madagascar, Comores, and Guadeloupe (7, pp. 90–104); and (2) holding the beans in an electric oven at 45° C. at high humidity until flexible.

There were 14 combinations of treatments each of which was replicated 4 times. Each replicate consisted of about 60 beans. After sweating, all the samples were dried on racks to approximately 35 percent of the fresh weight and then conditioned in tightly closed wooden boxes for approximately 5 months. Figures 1, 2, and 3 show the type of electric oven used for sweating, the removable screen racks for indoor drying, and a conditioning box for storage.

The factors used to evaluate the different curing methods statistically were: Degree of splitting, time taken for sweating and drying, mold development, vanillin content, and phenol value. Likewise, the samples were judged for aroma, crystallization, color, and flexibility by three persons experienced in the handling of vanilla.

#### DEGREE OF SPLITTING

The percentages of vanilla beans split by the different killing treatments and sun sweating were as follows: Scratching, 12.8; oven, 12.9; water at 80° C., 23.2; water at 65°, 2.65; freezing, 30.7; ethylene, 47.5; and sun, 62.5. The first two treatments were definitely superior. After oven sweating, the order was the same for the first five treatments (9.9, 16.2, 19.5, 21.7, and 26.2 percent), while in the last two treatments the percentages were interchanged—sun 27.6 and ethylene 35.7. The dif-

Appreciation is expressed to "La Cooperativa de Cosecheros de Vainilla de Puerto Rico" for the loan of some of the beans used in this and the following experiments.
 Italic numbers in parentheses refer to Literature Cited, p. 16.

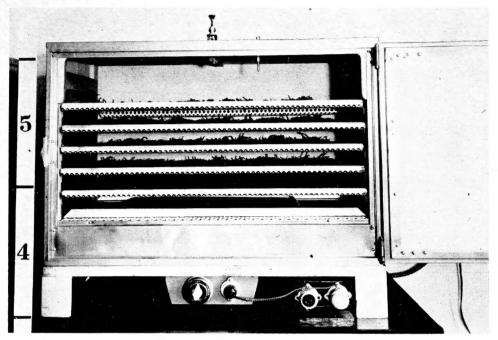


FIGURE 1.—Electric oven for sweating vanilla beans. Oven sweating involves less labor and decreases mold infection.

ference between the first two treatments was not statistically significant.4

Oven sweating was highly superior to sun sweating when the beans were killed in the sun and somewhat superior when they were killed in ethylene gas. The other differences, although in favor of oven sweating, were not significant.

#### SWEATING AND DRYING TIME

The rank of the killing treatments in average number of days necessary when followed by sun sweating was: Scratching 5.3, oven 8.6, freezing 14.9, ethylene 15.6, water at 65° C. 16.8, sun 17.2, and water at 80° 17.4. The differences among the first three treatments were highly significant. With oven sweating, the order in number of days was: Scratching 5.8, oven 6.9, ethylene 11.8, freezing 13.8, water at 80° 14.3, water at 65° 14.7, and sun 16.7 days. With the scratching treatment, significantly less time was required to complete sweating and drying than with the oven treatment, and both were markedly superior to the other treatments. Oven sweating was markedly superior to the sun method when the killing procedures used were oven, water at 80°, water at 65°, and ethylene, and was significantly superior when freezing was used.

#### MOLD DEVELOPMENT

With the various killing treatments followed by sun sweating, the percentage of moldy beans increased in the following order: Water at 80° C., 0.3; freezing, 0.4; water at 65°, 2.2; sun, 7.4; scratching, 10.1;

 $<sup>^4</sup>$  As the results obtained in splitting varied from percentages less than 25 to more than 50 they were coded on an angular basis, as suggested by Bliss (15, pp. 378–384), and the statistical analysis was made from the resulting figures.

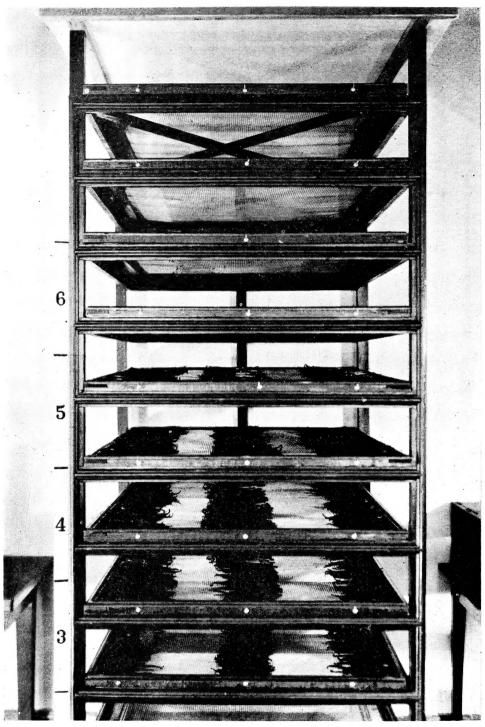


Figure 2.—Removable screen racks used for the indoor drying of vanilla beans to the desired weight after sweating.

oven, 10.3; and ethylene, 14.9. The first three treatments did not differ from each other significantly but all were significantly superior to the other four treatments. With oven sweating, the percentages were: Freezing, 0; water at 65°, 0.1; ethylene, 0.6; water at 80°, 0.9; oven, 3.5; sun, 4.7; and scratching, 6.8. The first four treatments resulted in

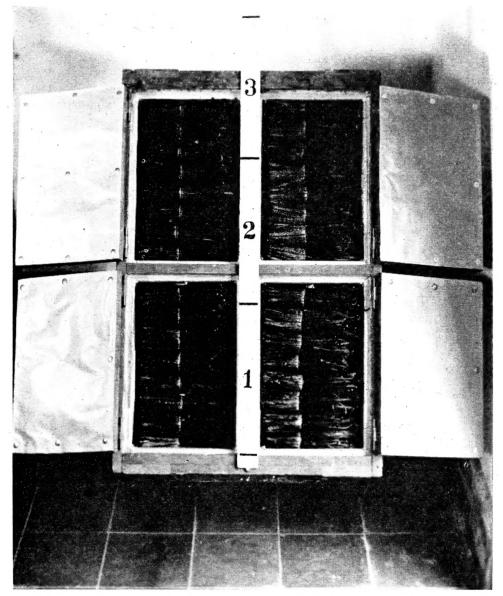


FIGURE 3.—Wooden box lined with tin and wax paper for conditioning and storage of vanilla beans.

practically the same percentage of mold development, but were superior to the last three. Oven sweating was decidedly superior to sun sweating when oven killing, sun killing, ethylene killing, or scratching was used, and somewhat superior when water at 65° was used. The other differences, although generally in favor of oven sweating, were not significant.

#### VANILLIN CONTENT

Vanilla beans that had been scratched, oven-killed, or ethylenetreated and then sun-sweated or oven-sweated generally had a high vanillin content. Beans killed in the following ways and then sun-sweated had the following percentages of vanillin: Scratching, 3.06; oven, 2.89; ethylene, 2.89; freezing, 2.59; water at 65° C., 2.57; water at 80°, 2.55; and sun, 2.47. The first treatment gave more vanillin than the second and third, but the difference was not statistically significant. The ethylene and oven treatments were significantly superior to the sun treatment in resulting vanillin content, but there was no significant difference between the results when the ethylene, oven, freezing, or the two hot-water treatments were used.

Beans subjected to the various killing treatments and then ovensweated contained the following percentages of vanillin: Water at 65° C., 3.08; oven, 2.89; scratching, 2.80; water at 80°, 2.77; freezing, 2.67; ethylene, 2.62; and sun, 2.37. The results of the first four treatments were not significantly different, but the results obtained with water at 65° were significantly superior to those with the freezing and ethylene treatments. Results with the oven-sweating method were markedly superior to those obtained with sun sweating only when the killing method with water at 65° was used, there being no statistically significant differences among the results with the other treatments.

#### PHENOL VALUE

The phenol value is a measure of the concentration of compounds having a hydroxyl group on an aromatic nucleus. Therefore, in vanilla extract, this value will include hydroxy compounds such as tannins, as well as vanillin. On the assumption that this phenol value (5)is a criterion of quality, the killing treatments when followed by sun sweating ranked in order of decreasing phenol value as follows: Scratching 8.46, ethylene 8.04, sun 7.99, oven 7.78, freezing 7.77, water at 65° C. 7.67, and water at 80° 7.56. The differences among the first three were not significant, but scratching was significantly superior to the other treatments. There was no significant difference among the other six treatments.

When the oven method of sweating was used the phenol value was: Scratching 9.62, freezing 8.54, ethylene 8.44, sun 8.36, oven 7.96, water at 80° 7.87, and water at 65° 7.84. In this series scratching was markedly superior to all other methods. There was no significant difference between freezing, ethylene, and sun killing or between sun, oven, and the two hot-water treatments. Oven sweating was decidedly superior to sun sweating when the scratching and freezing treatments were used, there being no difference among the others.

### ORGANOLEPTIC TESTS

Observation of the samples showed that with sun sweating only the beans killed by scratching yielded abundant vanillin crystals, while the other treatments gave no crystals. With oven sweating, all treatments gave some crystallization, scratching and freezing yielding abundant crystals. As to color, all samples were dark brown except those killed by freezing, which were reddish brown; scratching resulted in light-brown, woody stem ends. All of the samples ranked medium in flexibility, except the following: The scratched beans were low and the sun-killed beans high in flexibility; beans killed by freezing were high in flexibility when sun-sweated and very high in flexibility when oven-sweated. The aroma in all treatments was generally agreeable, but those lots which had had a high incidence of mold had a fermented aroma. The beans killed by the three processes ethylene gas, exposure to the sun, and scratching with a pin had the most well-developed aroma, while the frozen beans were characterized by the most suave vanillalike aroma. That of the oven and hot-water killed beans was less developed.

#### EVALUATION OF METHODS

In general all of the curing methods gave a satisfactory product, but the killing treatment involving scratching the beans with a pin appeared to be superior in that it resulted in a low degree of splitting, required a short time for sweating and drying, and gave high vanillin and phenol values and a well-developed aroma. However, it seemed to produce a high susceptibility to mold and a poor flexibility in the stem ends, which detracted much from their appearance. latter conditions, as well as the short time required for beans so treated to dry, were due to the fact that the beans lost water rapidly as a result of the scratches. A modified scratching method not extending to the stem end of the bean might obviate the lack of flexibility in that region. The high incidence of mold infection in these beans was undoubtedly due to inoculation of the wound with the pin, as well as to opening the surface to further contamination. General cleanliness and proper antiseptics which would not interfere with the aroma and flavor of the beans could probably be used to avoid an excessive degree of mold.

Only a few of the oven-killed beans split and they needed only a relatively short time for sweating and drying and were high in vanillin content, but a high percentage of them were moldy and their phenol

value was low.

The beans killed by freezing had practically no mold and a very suave aroma. However, they ranked medium in other respects. A practical advantage of this procedure is that the beans can be picked at the optimum stage of maturity and kept in a refrigerating unit un-

til enough of them accumulate to make curing worth while.

The ethylene treatment acted more as a maturing agent, as shown by the increase in vanillin content and phenol value and the greater development of aroma in the beans. It is not, however, a very effective killing treatment, as shown by the excessive degree of splitting, the rather long time needed for the beans to sweat and dry, and the high incidence of mold development. Therefore, if ethylene is to be used, it should be followed by a more effective killing treatment.

There was no significant difference in any of the factors of evaluation between beans killed in water at 80° C. and in water at 65°. Beans sweated in the oven were generally better than those sweated in the sun with respect to time for sweating and drying and mold development, the short time for the former being due to more regular and continuous heating and the low mold development being due to the less

favorable conditions for contamination. According to the other factors of evaluation oven-sweated beans were better than the sunsweated in only some of the treatments.

## EFFECT OF MATURITY OF BEANS ON QUALITY

In order to observe the effect of maturity upon the quality of the cured product, vanilla beans of three degrees of maturity were subjected to a hot-water killing process, sweated at 65° C., dried at room temperature to the desired weight, and conditioned for 6 months. The beans used were of three degrees of maturity, whole green, whole blossom-end-yellow, and split blossom-end-chocolate.

On examination of the cured beans it was found that the vanillin crystallization on the surface was greatest in the split blossom-endchocolate beans and decreased with maturity in the other samples. The color was blackest in the least mature beans and the whole blossom-

end-yellow beans had the most pleasing aroma.

The intensity of reddish-brown color and the total solids, vanillin, and resin contents of the beans were found to increase consistently with their degree of maturity. The extract from the blossom-end-yellow beans was classified as best in aroma. It should be mentioned, however, that when the beans are completely chocolate-colored before curing the resulting vanillin content after curing has been observed to be lower. Thus, in a series of experiments carried out during one curing season, the average vanillin content of all beans less mature than the chocolate stage was 3.49 percent, while that of chocolate-colored beans was only 2.68 percent. The loss of vanillin from chocolate beans was very likely due to volatilization of free vanillin during the early stages of the curing process. It will be seen in a later section that a considerable part of the vanillin in chocolate beans is free while that in less mature beans exists as the glucoside.

#### RELATION OF MOISTURE CONTENT TO QUALITY

Curing is accompanied by a loss of moisture throughout the process. The amount of moisture left in the beans influences their appearance and flexibility. A relatively high percentage of moisture is desirable and produces higher returns, as the beans are sold by weight. It is important, therefore, for the curer of vanilla to control moisture losses during curing. A series of experiments was carried out to show the relation of moisture content to quality of vanilla beans (3).

It was found that regardless of the killing treatment and the final moisture content of the cured beans, the total loss in weight during curing plus the water left in the cured product was from 79.7 to 81.1 percent of the original weight for whole green beans, and from 79.0 to 80.7 percent of the original weight for blossom-end-yellow beans. These figures represent approximately the average moisture content of these types of uncured beans. Therefore, weight loss, other than that due to loss of water, was found to be negligible.

Moisture losses in curing occurred principally during the sweating and drying treatments. Weight losses also occurred during the conditioning or final phase of the processing, during which vanillin crystallizes on the surface of the beans and the characteristic vanilla aroma

gradually develops.

There was an appreciable loss in weight in the beans during the first 3 months of conditioning. The loss in weight was found to be negligible, however, during the last 3 months, reaching not more than 1.3 percent. It is apparent that the moisture content approaches a steady value during conditioning and that the loss in weight during this period is generally greatest in the beans containing the most moisture.

It was found that the moisture left in cured beans affected the physical appearance and aroma of the beans in different curing treatments but that it did not have any significant effect on the phenol value. In all treatments, beans with moisture contents varying from 50 to 54 percent had a fermented aroma generally lacking in suavity and development. Beans with a moisture content of 31 to 34 percent had a well-developed, suave aroma and a high degree of flexibility, while those containing 24 to 27 percent moisture had a more developed and suave aroma but little flexibility. Beans with the lowest moisture content were generally dark in color and had much vanillin crystallization except when sun-cured, in which case no crystals appeared. Beans killed by freezing were reddish and were characterized by great flexibility, even when the moisture content was low.

No appreciable molding occurred in any of the treatments when the beans contained 24.2 to 26.5 percent moisture or in the beans having 30.7 to 50.5 percent moisture when subjected to hot water or freezing. As in previous experiments, beans subjected to the ethylene and sun killing treatments developed more mold than did those subjected to

hot water or freezing.

#### NOMOGRAPH FOR MOISTURE CALCULATIONS

The nomograph (fig. 4) shows the weights to which 100-pound lots of beans of different moisture contents should be reduced during curing to obtain a given moisture content in the end product (3). example, starting with beans of an assumed original moisture content of 80 percent to be cured to 30 percent final moisture, run a straight line from point 80 on left axis to point 30 on right inclined axis. Read off on center axis final weight of beans after curing including condi-To obtain the weight to which the beans should be dried before conditioning, the loss during conditioning should be added to that obtained from the chart. The loss during conditioning depends principally on the moisture content of the beans at the beginning of conditioning, the number and length of times the beans are examined, and the amount of wiping to remove molds. However, for approximate purposes, the following losses may be assumed: For 30 to 40 percent final moisture in the cured product. 4 to 7 pounds of each 100 pounds of fresh beans, and for 20 to 30 percent moisture, 1 to 3 pounds.

#### CHEMISTRY OF VANILLA CURING

#### GLUCOSIDES AND ENZYMES

The characteristic odor of vanilla is due to a mixture of oleoresins and vanillin formed in the beans during the curing process. Gobley, in 1858, according to Chalot (7, p. 141), was the first to isolate vanillin. Lecomte, in 1913 (12), suggested that the glucoside coniferin would give rise to coniferyl alcohol and this in turn would be oxidized to

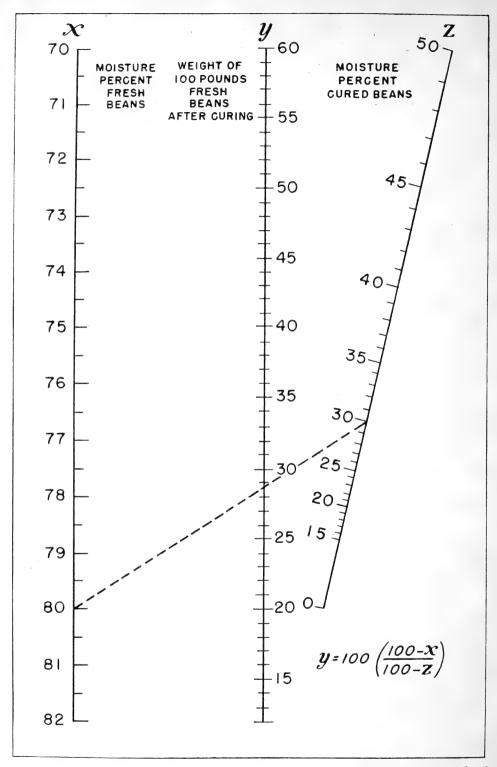


FIGURE 4.—Nomograph showing the three variables: X, Moisture content of fresh vanilla beans in percent, fresh basis: Y, weight to which each 100 pounds should be reduced during curing; and Z, moisture content of cured beans in percent, cured-weight basis. See example given in the text.

vanillin. However, this glucoside has never been isolated from vanilla beans. Goris, in 1924 (10), concluded that three glucosides were present in the beans: glucovanillin, which he isolated; glucovanillic alcohol, which was characterized by its product of hydrolysis, vanillic alcohol; and an unisolated glucoside which produced on hydrolysis an ether of strong and suave aroma. He suggested that the glucovanillic alcohol would produce the glucovanillin by oxidation and this in turn would hydrolyze to vanillin. Other constituents found in some types of beans are esters and other derivatives of cinnamic acid (8), piperonal, or heliotropine (9), and anisyl alcohol and derivatives of anisic acid (17).

#### GLUCOVANILLIN

The vanilla bean is composed essentially of a central portion containing the seeds and placental tissue, surrounded by a fleshy part, or ovary wall. Figure 5 shows an uncured pod in longitudinal and cross sections.

Studies of the glucovanillin content of the beans were carried out by the author (2). A definite gradient in glucoside concentration, increasing from the stem to the blossom end of the beans, was found. This accounts in part for the observed fact that vanillin crystals form during curing principally on the blossom end of the bean, as shown in figure 6. It was calculated that the glucovanillin was distributed as follows: 40 percent in the blossom end, 40 percent in the middle, and

20 percent in the stem end.

Maturation of vanilla beans is indicated by a yellow coloration that develops in the blossom end of the pod. As ripening proceeds, this coloration spreads along the entire bean and is usually accompanied by longitudinal splitting. The beans finally acquire a chocolate color. Only traces of vanillin have been found to be present in whole green beans and in split, blossom-end-yellow beans, thus showing that there was only a slight hydrolysis of glucovanillin up to the blossom-endvellow stage of maturity. The vanillin content of chocolate-colored beans, however, was found to be very high, showing that the glucoside was hydrolyzed as a result of the natural ripening process. The total glucovanillin content of treated glucoside extracts was about 8.8 percent on a dry basis and apparently more was formed during ripening from the blossom-end-vellow to the chocolate stage. The increase in reducing sugars and other substances giving a similar reaction with Fehling's solution from the vellow to the chocolate stage was found to be greater than could be accounted for on the basis of glucovanillin hydrolysis alone.

### $\beta$ -GLUCOSIDASE ACTIVITY

The presence of a hydrolytic enzyme in vanilla beans was shown by Lecomte (12) and further experiments on its action were carried out by the author (2). A wide variation in hydrolytic or  $\beta$ -glucosidase activity was found among beans of various stages of maturity. A negligible amount of active enzyme was present in uncured green beans, more was present in blossom-end-yellow beans, and the most in split, blossom-end-yellow beans. No active  $\beta$ -glucosidase was found in the central seed portion and placental tissue of the beans, all of the enzyme being located in the fleshy part or thick wall of the pods. Since it was

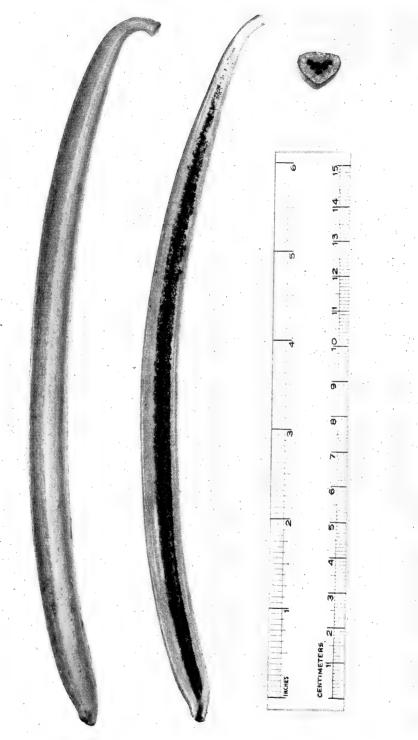


FIGURE 5.—Uncured beans of *Vanilla fragrans* showing the entire pod and longitudinal and cross sections. An active  $\beta$ -glucosidase which produces the hydrolysis of the vanillin-containing glucoside was found in the outer, fleshy portion or the ovary wall. The glucoside was distributed throughout the outer portion and the central portion, which includes seeds and placental tissue.

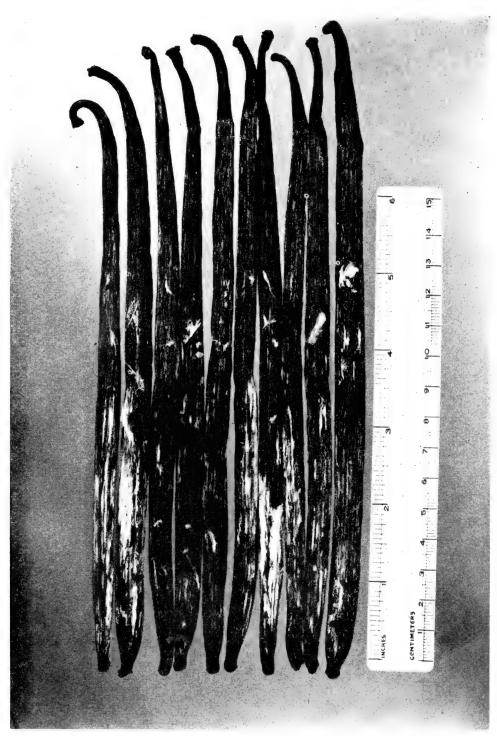


Figure 6.—Cured vanilla beans showing the occurrence of vanillin crystals on the lower two-thirds portion of the blossom end of the pods. The glucovanillin was found to be present in largest amount in the blossom end of the pod and decreased gradually toward the stem end.

found that some glucovanillin exists in the central portion of the bean where there is no enzyme, it must be concluded that during curing either this glucovanillin diffuses outward with the water and is hydrolyzed upon reaching the outer wall, where the enzyme is located, or the enzyme diffuses throughout the whole pod.

Storage of harvested green beans showed a gradual increase in enzyme activity with maturation. However, storage beyond 10 days, during which the beans became chocolate in color for half their length, resulted in partial inactivation of the enzyme. The  $\beta$ -glucosidase in the latter beans, however, was found to be reactivated during curing to a level comparable with original activity.

In beans cured in the green stage, glucosidase activity was found to be very low throughout the curing process, and the enzyme was not greatly activated by exposure to a temperature of 45° C. The curing of green beans usually results in an inferior product, and the foregoing indicates that the poor results are due, at least in part, to the low activity level of the enzyme. On the other hand, the fact that such beans are of passable quality confirms previous observations (4) that the quality of vanilla beans is not entirely due to the presence of vanillin.

#### THE PEROXIDASE SYSTEM

The presence of a peroxidase in vanilla beans was determined  $(1, pp. \bar{2}-5)$  and its fate during the curing process studied (4). It was found that this peroxidase was definitely resistant to various methods of wilting the beans and survived a lengthy conditioning period very The preparation of a crude peroxidase from green vanilla beans made it possible to show that this enzyme could oxidize vanillin in vitro. It was apparent, therefore, that vanillin, a flavoring material present in the cured beans, is oxidizable under certain conditions by an enzyme that also occurs in the beans. Whether such oxidation actually takes place in the tissue of the bean depends doubtless on many factors, but obviously it cannot take place unless a peroxide is also present. The presence of a peroxide in the respiring tissues of green beans was to be expected, but not necessarily in the dead tissue of cured or partly cured beans. Nevertheless, qualitative tests for peroxide were positive not only with green beans but also with beans that had completed the sweating period and with others that had been The details of such tests were reported by Balls and completely cured. Arana (4). Peroxidase may, therefore, play an important part in the oxidation that takes place during curing. Vanillin, once formed from the parent glucoside, might be further oxidized, with the production of quinone bodies of more complicated structure and presumably of This would explain the long-delayed development different aroma. of the characteristic vanilla aroma and the fact that the finest aroma is not necessarily accompanied by a high vanillin content. On the other hand, the present emphasis on peroxidase must not be taken as indicating a belief that it is the only oxidative enzyme of importance involved, for, at least in green beans, qualitative evidence of the presence of an oxidase was obtained (1, 12).

#### RESPIRATION OF VANILLA BEANS

In estimating the rate of over-all oxidation in vanilla beans, the carbon dioxide given off was measured in a Truog apparatus (16).

The measurements were made of the beans before and after subjec-

tion to different killing methods (4).

Dipping in warm water, scratching the surface with a needle, or exposure to ethylene gas produced an immediate rise in the rate at which carbon dioxide was given off. A reasonable explanation of the effect is that an injury to the outer tissues of the fruit is responsible for the increased rate of respiration. It is, therefore, probable that these methods of killing have as their basis an initial acceleration of the rate of oxidation in the tissue. However, the reverse effect was observed when the beans were frozen; on thawing there was a decrease in the rate of respiration.

The respiration rate was determined at various intervals during the curing process in beans killed with ethylene gas, with hot water, and by freezing (4). The respiration rate of a lot of beans subjected to ethylene gas increased in 1 hour after killing from 66 to 102 milligrams per kilogram per hour, in one killed in hot water the rate increased from 79 to 123, and in another killed by freezing it decreased from 79 to 73 milligrams per kilogram per hour. In all cases after the wilting the rate of carbon dioxide evolution decreased until during the conditioning period the rate was practically negligible.

#### EFFECT OF GASES AND HYDROGEN PEROXIDE ON VANILLIN FORMATION

At the suggestion of A. K. Balls some work was done on the effect of hydrogen sulfide, oxygen, nitrogen, and hydrogen peroxide on the formation of vanillin. It was expected that the inactivation of peroxidase by hydrogen sulfide might lead to a higher vanillin content because none would be oxidized by the enzyme. The oxygen was expected to accelerate oxidative changes and the peroxide to accelerate the peroxidase action.

The results showed that the use of oxygen, nitrogen, or hydrogen peroxide led to no significant change in the vanillin content or phenol value. The hydrogen sulfide treatment resulted in a lower vanillin content and a retarded development of the brown color during curing.

#### **SUMMARY**

An experiment was carried out to evaluate statistically several commercial vanilla-curing methods and others devised at the laboratory. In general all the methods gave a satisfactory product. The Guadeloupe killing treatment, which involves scratching the beans with a pin, appeared to be superior in all criteria of evaluation except appearance and susceptibility to mold. The beans killed by freezing had practically no mold and a very suave aroma but ranked medium in other respects. The ethylene treatment acted more as a maturing than as a killing agent. Oven sweating was found to be superior to sun sweating as to time required for sweating and drying, and mold development.

The intensity of reddish-brown color and the total solids, vanillin, and resin contents of the beans were found to increase consistently

with the degree of maturity of the beans.

Weight loss, other than that due to loss of water, was negligible during curing. Moisture content approached a steady value during

the first 3 months of conditioning. The optimum moisture content of cured beans was considered to be from 30 to 35 percent. A nomograph is given showing the weights to which 100-pound lots of beans should be reduced during curing to obtain a given moisture content in the end product.

Studies on the glucovanillin content and its distribution in the beans are discussed. The distribution and action of the  $\beta$ -glucosidase

which hydrolyzes the glucovanillin is also pointed out.

The presence of a peroxidase system and its role in the curing process was considered.

The rate of over-all oxidation in the beans after various killing treatments and during the curing process was determined by measuring the

carbon dioxide given off.

Preliminary experiments were carried out on the effect of hydrogen sulfide, oxygen, nitrogen, and hydrogen peroxide on the formation of vanillin. The hydrogen sulfide treatment resulted in lower vanillin content and retarded development of brown color.

#### LITERATURE CITED

(1) Arana, F. E. 1940. Chemistry of Vanilla. Puerto Rico (Mayaguez) Agr. Expt. Sta. Rpt. 1939: 2-14, illus.

(2) ARANA, F. E.

1943. ACTION OF A  $\beta$ -GLUCOSIDASE IN THE CURING OF VANILLA. Food Res. 8: 343–351, illus.

(3) ARANA, F. E., and KEVORKIAN, A. G.

1944. THE RELATION OF MOISTURE CONTENT TO QUALITY OF VANILLA BEANS. (In press.)

(4) BALLS, A. K., and ARANA, F. E.

1941. THE CURING OF VANILLA. Indus. and Engin. Chem. 33: 1073-1075, illus.

(5) Balls, A. K., and Arana, F. E.

1941. DETERMINATION AND SIGNIFICANCE OF PHENOLS IN VANILLA EXTRACT.
ASSOC. Off. Agr. Chem. Jour. 24: 507-512.

(6) Balls, A. K., Kevorkian, A. G., and Arana, F. E.

1942. PROCESS FOR CURING VANILLA BEANS. U. S. Patent No. 2,274,120. U. S. Patent Office, Off. Gaz. 535: 802.

(7) CHALOT, C., and BERNARD, U.

1920. CULTURE ET PRÉPARATION DE LA VANILLE. 216 pp., illus. Paris.

(8) GNADINGER, C. B.

1925. IDENTIFICATION OF SOURCES OF VANILLA EXTRACTS. Indus. and Engin. Chem. 17: 303-304.

(9) GNADINGER, C. B.

1926. PIPERONAL IN VANILLA EXTRACT. Indus. and Engin. Chem. 18: 588-589.

(10) Goris, M. A.

1924. SUR LA COMPOSITION CHIMIQUE DES FRUITS VERTS DE VANILLE ET LE MODE DE FORMATION DU PARFUM DE LA VANILLE, [Paris] Acad. des Sci. Compt. Rend. 179: 70–72.

(11) KEVORKIAN, A. G.

1940. VANILLA-PROCESSING STUDIES. Puerto Rico (Mayaguez) Agr. Expt. Sta. Rpt. 1939: 14-22.

(12) LECOMTE, H.

1913. FORMATION DE LA VANILLINE DANS LA VANILLE. Agr. Prat. des Pays Chauds 13 (2): 3-14.

(13) Mallory, L. D., and Walter, K.

1942. MEXICO'S VANILLA PRODUCTION. U. S. Dept. Com., Foreign Commerce Weekly 7 (11): 8–10, 23, illus.

(14) Pennington, C. F.

1938. VANILLA BEAN PROCESSING STUDIES. Puerto Rico (Mayaguez) Agr. Expt. Sta. Rpt. 1937; 24–26.

- (15) SNEDECOR, G. W.
  1940. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. Ames, Iowa.
- (16) Truog, E.

  1915. Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrimetric method. Indus. and Engin. Chem. 7: 1045–1049, illus.
- (17) Walbaum, H.
  1909. das vorkommen von anisalkohol und anisaldehyd in den früchten
  der tahitivanille. Wallachs Festschrift, 1909: 649–653.

